
In The Name Of
GOD

Cell Free DNA As a Diagnostic Marker

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Content

What Is The Cell Free DNA?

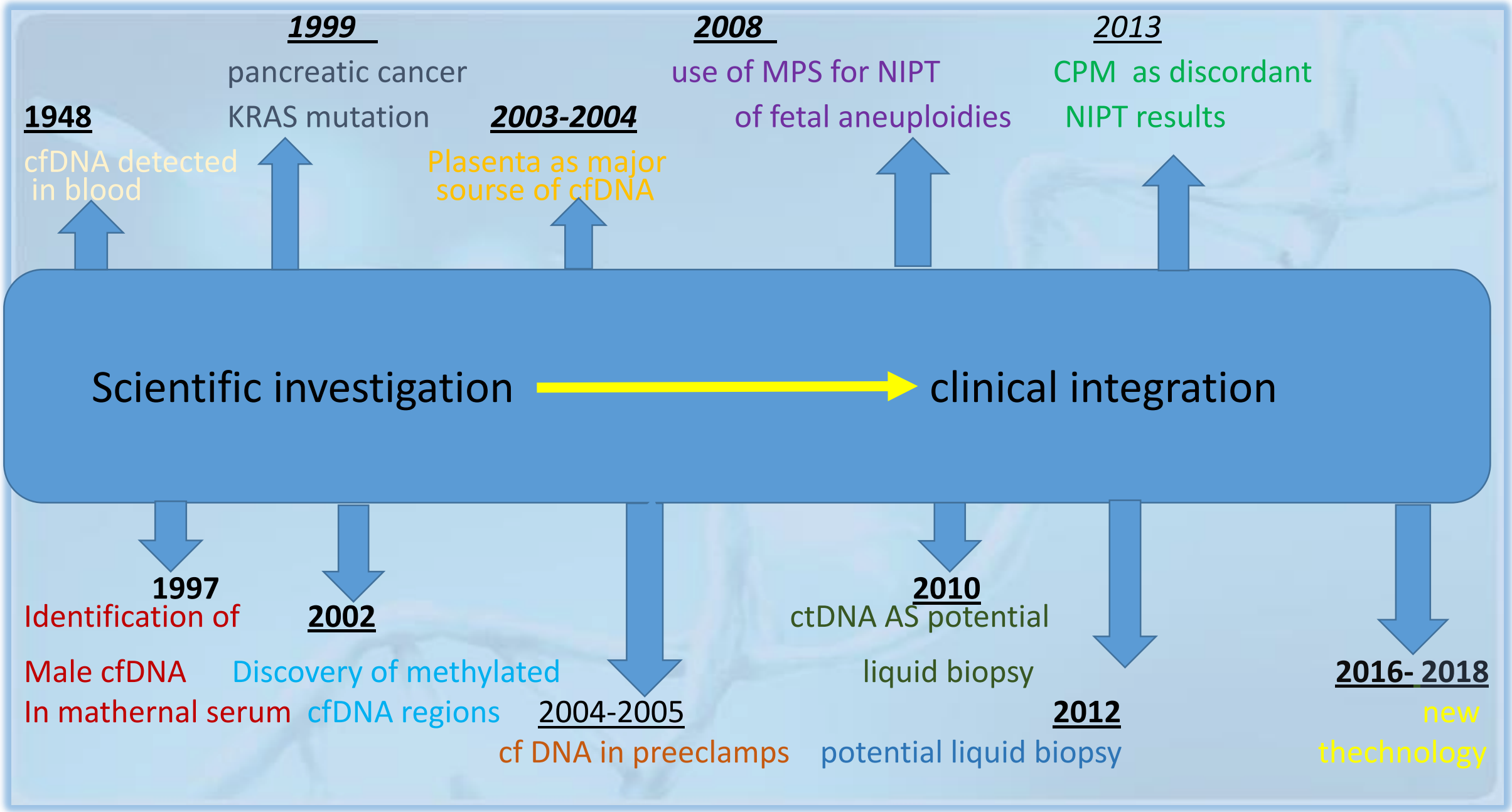
- double-stranded nucleic acid fragments
- Inter blood stream during apoptosis or necrosis
- Cleaned up by macrophages
- is called ctDNA if originates from a tumor clone
- around 170 bases in length
- half-life of about two hours[1]

What Is The Cell Free DNA?

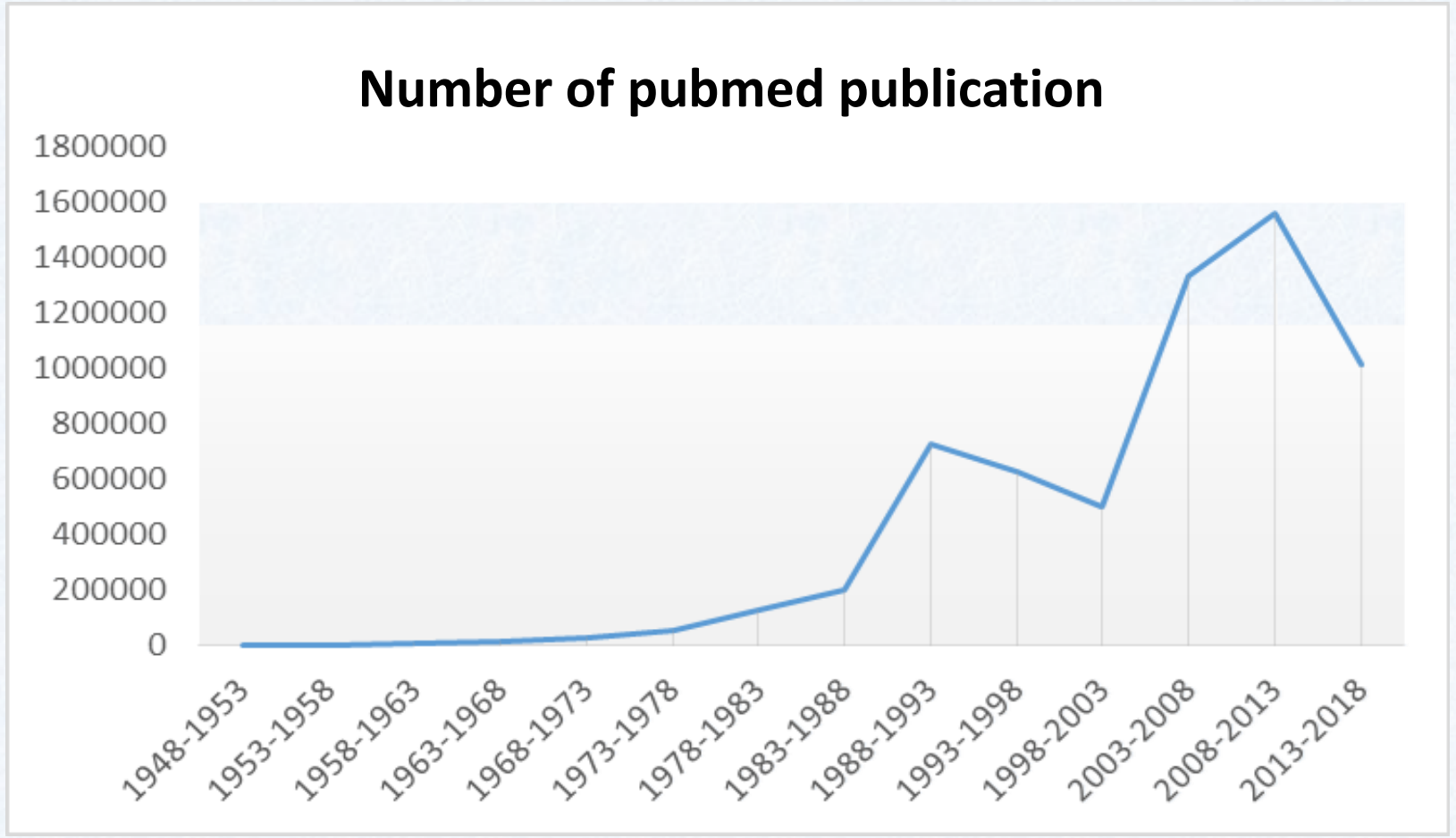
- can be further subcategorized to:

✓circulating mitochondrial DNA (mtDNA)

- Circulating nuclear DNA(nDNA)[20]
- lower molecular weight than genomic DNA


1948
cfDNA detected in blood
1999
pancreatic cancer
KRAS mutation
2003-2004
Plasenta as major sourse of cfDNA
2008
use of MPS for NIPT of fetal aneuploidies
2013
CPM as discordant NIPT results
Scientific investigation
clinical integration
1997
Identification of Male cfDNA In mathernal serum
2002
Discovery of methylated cfDNA regions
2004-2005
cf DNA in preeclamps
2010
ctDNA AS potential liquid biopsy
2012
potential liquid biopsy
2016- 2018
new thechnology

Historical Developement



Retrieved from www.ncbi.nlm.nih.gov/pupmed

What Is The Cell Free DNA?

Cf DNA detected in:

- Liquid biopsy

I. Plasma

II. Blood

III. Urea

IV. Cerebrospinal fluid

V. Peritoneal fluid

VI. Fetal

Solid biopsy

I. Solid tumor

What Is The Cell Free DNA?

liquid biopsy

fast and easy
not cover all the physiological cfDNA concentration range
ranges of the analysed samples close to LOD of those kits
 small intensity response
low reliability
few commercial kits are available[24]

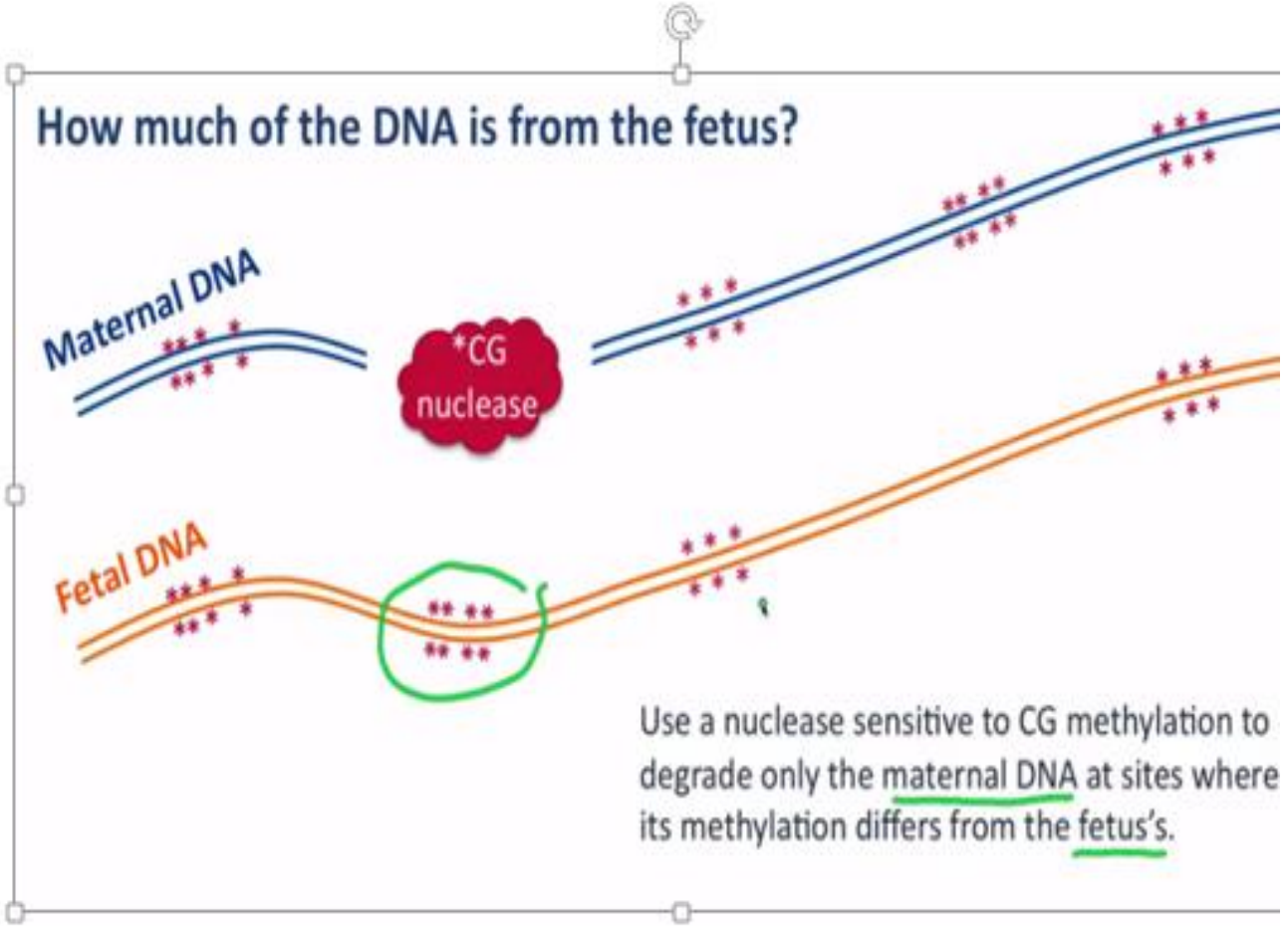
cfDNA In Plasma

- Drive from hematopoietic cells.
- Is fragmented by plasma nucleases
- Is realeased into plasma from tumor cells and can be detected there
- Depending on individual health and/or therapy status [2]
- in maternal plasma is a reflection of placental health and disease[3]

What is the cell free DNA?

- .Plasma

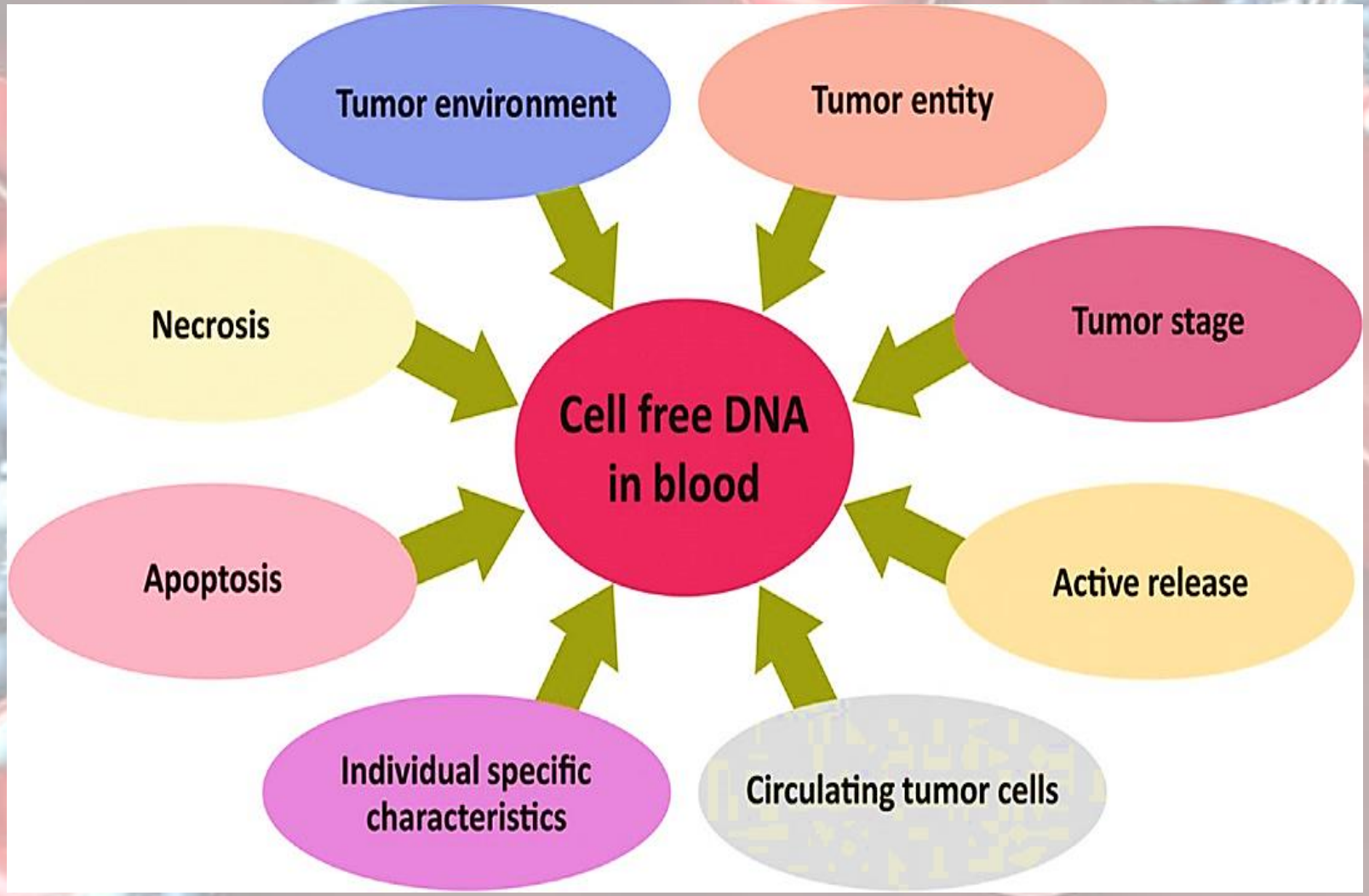
Professor Roise Redfield
The University of British



cfDNA IN BLOOD

- is a less invasive.
- are easy to obtain.[4]
- Normal concentration of cf DNA in healthy individuals varies from 0 to 100 ng/mL of blood, on average 30 ng/mL[14]

cfDNA in Blood



www.sec.gov

cfDNA in urea(ucfDNA)

- ultra-noninvasive sample over tissue and blood.
- Compared with blood, less is known about the its role
- tool for cancer screening, diagnosis, prognosis,
- monitoring of cancer progression and therapeutic effect[5]

cfDNA in cerebrospinal fluid

- reflected response to treatment or progressive disease
- serve as a diagnostic especially tumor drived cfDNA
- complemnts MRI and more sensitive than conventional cytology.[17]
- Using dig pcr for detect and NGS
- After emotional stress (ES) in brain tissue could be detected
- fetal cfDNA was found in the CSF of pregnant women[29]

Unique genetic profiles from cerebrospinal fluid cell-free DNA in leptomeningeal metastases of *EGFR*-mutant non-small-cell lung cancer: a new medium of liquid biopsy

Y S Li, B Y Jiang, J J Yang, X C Zhang, Z Zhang, J Y Ye, W Z Zhong, H Y Tu, H J Chen, Z Wang
... Show more

Annals of Oncology, Volume 29, Issue 4, 1 April 2018, Pages 945–952,
https://doi.org/10.1093/annonc/mdy009
Published: 15 January 2018

Abstract

Background

Leptomeningeal metastases (LM) are more frequent in non-small-cell lung cancer (NSCLC) with epidermal growth factor receptor (*EGFR*) mutations. Due to limited access to leptomeningeal lesions, the purpose of this study was to explore the potential role of cerebrospinal fluid (CSF) as a source of liquid biopsy in patients with LM.

Conclusion

CSF cfDNA could reveal the unique genetic profiles of LM and should be considered as the most representative liquid biopsy medium for LM in *EGFR*-mutant NSCLC.

cf DNA IN Peritoneal fluid

- is present in the overnight peritoneal effluent of stable CAPD patients.
- role in the diagnosis and prognosis of therapy-related peritoneal membrane degeneration.[16]

Fetal cf DNA

- potential markers for NIPT
- placental condition in pregnancy
- Realese into mother blood
- Undergoing apoptosis[32]
- Requires only sample from the mother

✓Revealed maternal metastatic disease, maternal mosaicism, placental mosaicism

✓adopted into clinical practice, due to positive insurance coverage[25]

clinical application

Pathological / psychological state	Qualitative and quantitative free DNA changes
Autoimmune diseases rheumatoid arthritis systemic lupus erythematosus systemic sclerosis primary Sjögren's syndrome	Increased free DNA level; Anomalous patterns of DNA typical for each disorder
Tumors	Increased free DNA level; Tumor DNA specific genetic and epigenetic changes
Transplantation (acute rejection)	Increased donor free DNA level; Increased recipient free DNA level
Trauma Sepsis Intensive care	Increased free DNA level
Acute states acute myocardial infarction acute mesenteric ishemia acute stroke acute pancreatitis	Increased free DNA level
Pregnancy	Increased free fetal DNA level; Increased free total DNA level; Fetal DNA specific changes

Raghu Aarthy(2015) Role of Circulating Cell-Free DNA in Cancers

Clinical Application

I. Cancer

- Used as a diagnoprognostic or predictive biomarker
- Provides valuable insights in tumor biologystic,.
- for developing new cancer-specific targets.
- clinical utility during and after treatment(ctDNA concentrations correlate with tumor size)[6]

Clinical Application

I. Cancer

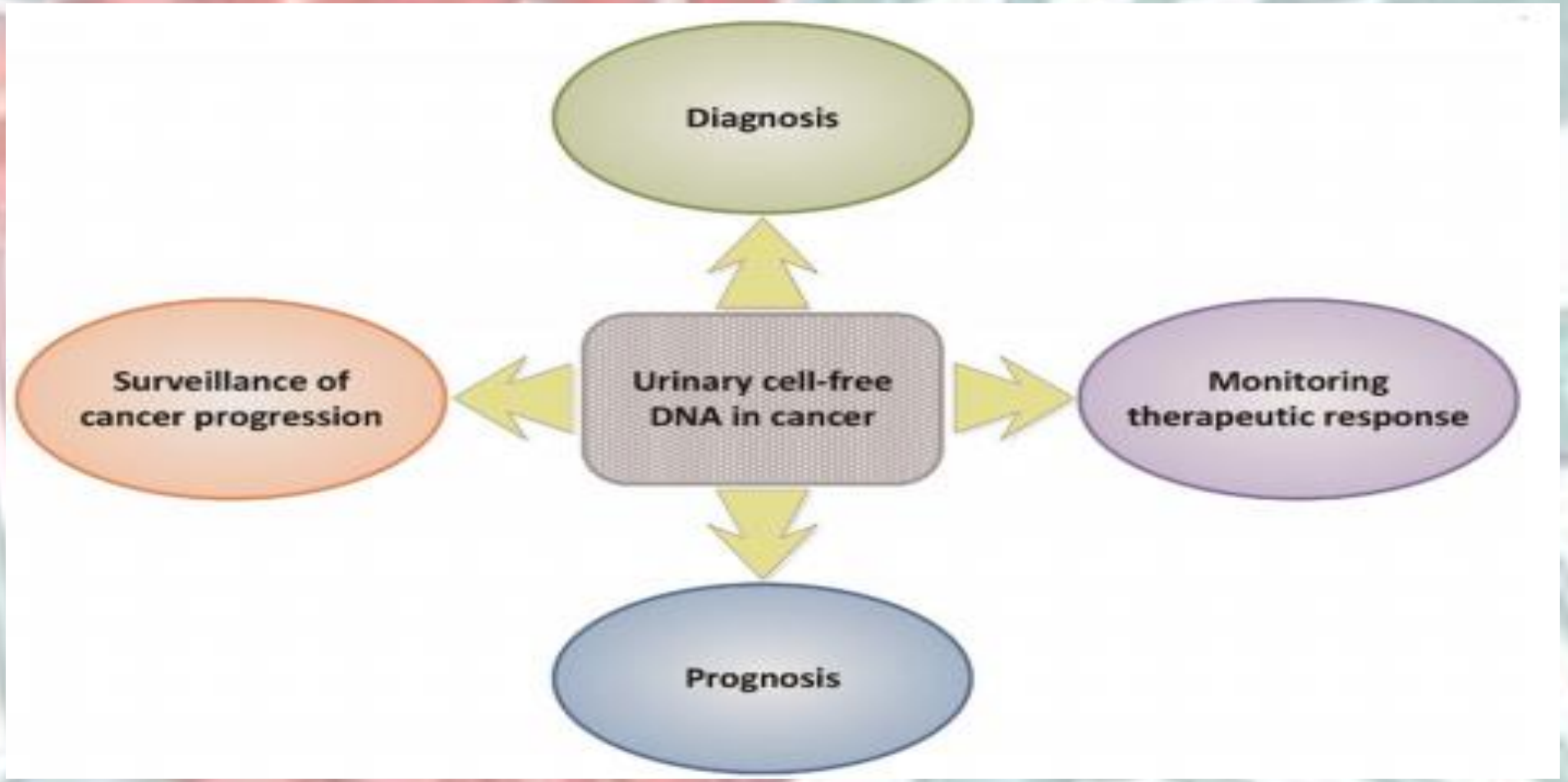
- enables new strategies for personalized cancer medicine[7]
- ucfDNA genetic alterations are mostly evaluated for urological cancers[8]
- Breast cancer metastasis suppressor-1promoter methylation in cf DNA isolated from plasma of non-small cell lung cancer patients provides prognostic information[12]
- cfDNA stimulate hormone receptor-positive breast cancer cell proliferation

by activating the TLR9-NF-κB-cyclin D1 pathway

- concentration correlated positively with the percent of cells in the G1 phase[13]

Clinical Application

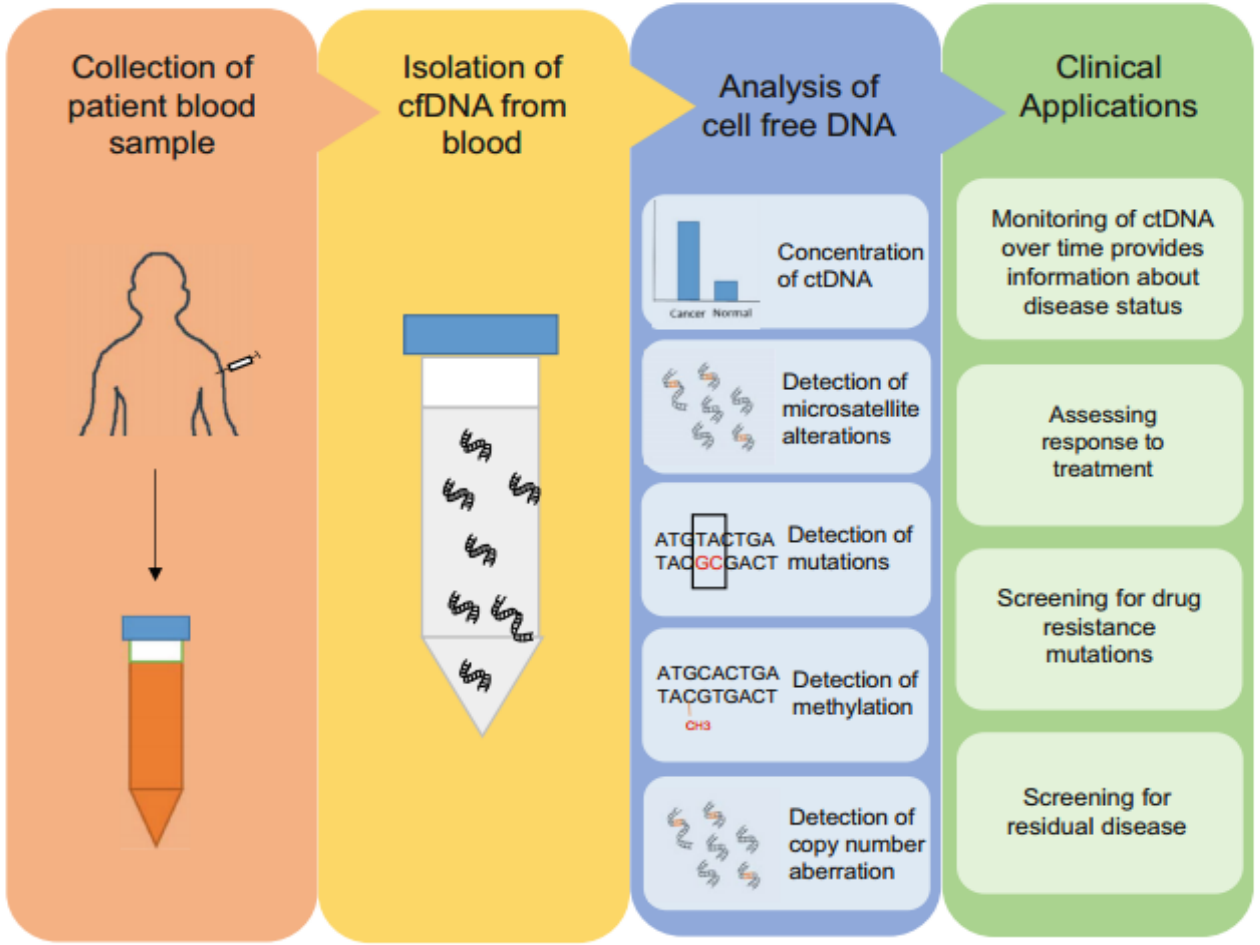
- I.CANCER



Tian Lu.(2017) Clinical applications of urinary cell-free DNA in cancer: current insights and promising future.

Clinical Application

Fig. 3 Clinical applications of circulating tumour DNA. Circulating tumour DNA is easily accessible as it is obtained from the peripheral blood of patients. It is being explored as a potential biomarker for cancer through the measurement of circulating tumor DNA concentration and detection of genetic alterations such as mutations, methylation, and microsatellite alterations. Monitoring these parameters in periodically collected blood samples would give information about disease progression and treatment response. It also allows screening for drug-resistance mutations, thereby aiding in treatment decision making. Minimal residual disease can be detected with the evaluation of cell-free DNA. *cfDNA* cell-freeDNA, *ctDNA* circulating tumor DNA



Raghu Aarthy(2015) Role of Circulating Cell-Free DNA in Cancers

Clinical Application

II.Spontaneous Abortion

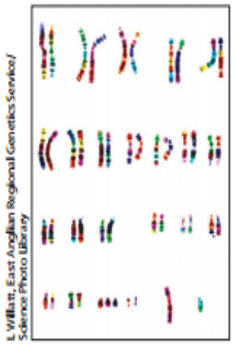
- potential markers for noninvasive monitoring during the pregnancy[9]
- increased in with spontaneous preterm birth.[10]
- FF cfDNA level was an independent and predictive factor for pregnancy outcome.[11]
- can be detected Chromosomal anomalies[12]

Clinical Application

II. Spontaneous Abortion

Cell-free DNA screening for trisomy is rolled out in Israel

The world's second largest health maintenance organisation is reimbursing women who have been referred for cell-free fetal DNA analysis by a consultant obstetrician. Anna Petherick reports.



L Willatt, East Anglian Regional Genetics Service/ Science Photo Library

More than 200 pregnant Israeli women have had the fetal DNA in their blood plasma screened for trisomy disorders such as Down's syndrome since July, when Clalit, a health maintenance organisation (HMO) that covers more than half the country's population, announced it would be offering one of the tests through its MOR Institute in Tel Aviv. Another of Israel's four HMOs, Maccabi, followed suit in August, making the country the world's first to roll out the test in this way. Depending on the details of their health insurance policy, Israeli women who have a high risk of carrying a fetus with Down's syndrome can expect reimbursement of up to 75% of the cost of the €1200 test.

The new policy is part of a suite of changes to the Israeli health-care system that will shift prenatal screening companies—employ one of two DNA sequencing strategies. Some of them randomly sequence DNA molecules in the plasma, while others use targeted sequencing, whereby they selectively amplify parts of the fetal genome of interest. Because the tests all provide a non-invasive means of screening for genetic abnormalities as early as 9 weeks into gestation, they have become hugely popular in a short period. Dennis Lo, a professor at the Chinese University of Hong Kong who originally identified fetal DNA

"Although highly accurate, cfDNA tests are not diagnostics, so a positive result still requires confirmation with amniocentesis..."

more commonplace developmental problems, such as pre-eclampsia. And although the tests can be done early in pregnancy, a turnaround of about 10 working days is usually required for the samples to find their way to the test providers' laboratory. The samples collected by staff at the MOR Institute, for example, will be mailed to California for analysis, and the results emailed back. Moreover, a variable percentage of the tests yield no result at all, often because the proportion of fetal DNA in the maternal plasma sample is insufficient. This outcome is more common among women with high body-mass indices than among women in the healthy range. It also varies between the different cfDNA tests: depending on the provider in question, as few as 1% of expectant mothers, or

Clinical Application

III.Forensic

- Cf DNA of a suitable length for standard DNA profiling are transferred during handling/touching[26]

Presence and potential of cell free DNA in different types of forensic samples

Mado Vandewoestyne, David Van Hoofstat, Aimée Franssen, Filip Van Nieuwerburgh, Dieter Deforce
Laboratory of Pharmaceutical Biotechnology, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium

PlumX Metrics

DOI: https://doi.org/10.1016/j.fsigen.2012.12.005

Article Info

Abstract | Full Text | Images | References | Supplemental Materials

Abstract

Extracellular or cell free DNA has been found to exist in many biological media such as blood and saliva. To check whether cell free DNA is present in the supernatant which is normally discarded during several DNA extraction processes, such as Chelex® extraction, DNA profiles of cell pellet and concentrated supernatant from 30 artificial case like samples and from 100 real forensic samples were compared. Presence of cell free DNA was shown in all investigated sample types. Moreover, in some samples additional alleles, not detected during analysis of the cell pellet, were detected, offering valuable information which would normally have been discarded together with the supernatant. The results presented here indicate that cell free DNA deserves further consideration since it has the potential to increase the DNA yield in forensic casework samples in general and in contact traces in particular.

Clinical Application

- Autoimmune disease

✓ SLE

- DNA structures that are targeted by auto-antibodies play a central role in SLE and that DNA-antibody complexes in the circulation are one of the hallmarks of SLE[14]
- it prove to be relevant to diagnostic and assessment of therapy[15]

Clinical application

- Myocardial infraction
- associated with the apoptosis and death of cardiomyocytes.
- is released following programmed cell death or acute cellular injury.
- detected in plasma from patients.[18]

Clinical application

Trauma

- correlates inversely to mortality, trauma severity, and post-traumatic complications.
- has received increasing attention in ICU patient as a result of truma.
- a marker of the patient's condition in the immediate emergency phase due to short half-life. [21]
- the innate immune response following aseptic injury.
- the systemic inflammatory response observed following trauma.

Clinical application

Trauma

may also give rise to coagulopathy by:

- platelet aggregation
- inhibition of fibrinolysis
- coagulation factor activation[22]

Quantification of cfDNA

Methods	Advantages	Disadvantages
Quantitative PCR	Wide choice in detection chemistry and reaction volume equates to flexible running costs	Fewer genes can be studied at a given time
	Larger number of samples can be analyzed	
	Large dynamic range	
	Relative measurement	
Digital PCR	High precision for better reproducibility for low-input target concentrations	Initial cost of equipment
	Higher precision for higher copy number variation analysis	Pre-amplification of target DNA on samples may result in biased amplification of input DNA
	Greater sensitivity for rare mutation detection	
	Absolute measurement eliminates need for standard curve	
Next-generation sequencing		
Targeted sequencing	Permits large number of genes to be studied in large number of samples (multiplexing possible)	Sensitivity depends on depth of sequence coverage
	Specific genomic region/genes can be analyzed	Possibility of detecting false positives
		Higher cost
Exome sequencing	Clinically relevant information from cell-free DNA point of view	Higher input material is required; higher cost implication

Raghu Aarthy(2015) Role of Circulating Cell-Free DNA in Cancers

Quantification of cfDNA

- 32P-labeled radioimmunoassays.
- Spectrophotometric determination
- Invitrogen’s Qubit® quantitation fluorometer highly accurate and sensitive detection
- commercial fluorescent SYBR® Gold stain
- Fluorometric PCR assay[7]

Quantification of cfDNA

MASS-Spectrometry

- MALDI-TOF MASS Spectrometry
- ESI-Mass spectrometry

Quantification and analysis of cf DNA

PCR

- Alu-based real time PCR
- Alu reduplicative elements
- signal amplification using fluorescence quantification.
- reliable, accurate and sensitive.
- normal control cfDNA <MI group
- ALU4 and ALU5 showed better sensitivity and compared with cTnI ,CK-MB,LDH

Alu-based real time PCR

- Alu sequences account for >10% of the genome and are abundant in blood
- Therefore, a potentially sensitive approach for the measurement of human cfDNA in blood.

Alu-based real-time PCR

ROC

to evaluate the predictive value of Alu-based real-time PCR[18]

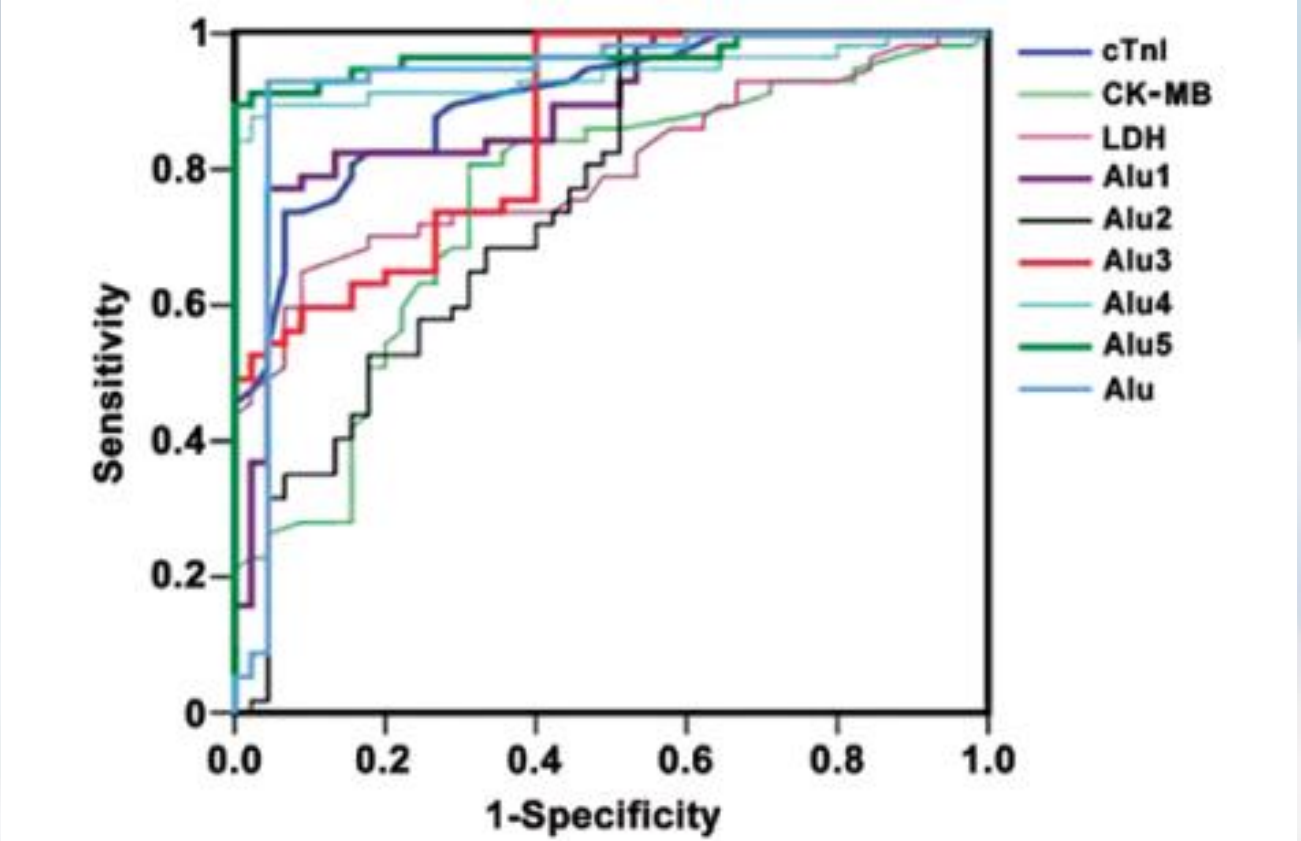


Figure 7. Receiver operating characteristic (ROC) curves of cardiac troponin I (cTnI), CK, creatine kinase MB (CK-MB) isoenzyme, lactate dehydrogenase (LDH), Alu1, Alu2, Alu3, Alu4, Alu5 and Alu. Alu4, Alu5 and Alu showed

Quantification of cfDNA

MALDI-TOF MASS Spectrometry

- accurate and sensitive high-throughput clinical diagnostic tests.
- precise determination of fetal SNP.
- noninvasive detection of fetal point mutations for βthalassemia.[19]

Quantification of cfDNA

ESI-Mass spectrometry

For example in HCC:

- sensitive for identification of mutations
- HPLC-ESI-MS-SOMA protocol used for screening
- SOMA assay combines:

✓PCR amplification

✓restriction digestion

✓Electrospray ionization mass spectrometry[7]

technical advances

- new assay :

✓low concentration range, around the LOD

✓signal amplification strategies:

❖ antibody conjugates

❖enzymatic amplification

❖sophisticate equipmen

- overcomes the limitations of the commercial kits
- without complex designs

technical advances

- NGS
- Sequensing TM
- Nanothechnology:

✓A plasmonic nanosensor with inverse sensitivity

✓A Versatile Nanowire Platform

✓electroactive conducting polymer nanowire platforms

- The value fluorimetry and spectrophotometry(nanodrop)

Next generation sequensing

- comprehensive of genomic targets from a single sample
- it obviates the need for repeat invasive tissue biopsies[27]
- Very high ability
- Massively parallel
- Cost lower than sanger sequensing
- chromosomal and microdeletion disorders through maternal blood screening.
- Prenatal genetic carrier screening

Next generation sequensing

Disadvantage

- a lot of raw information
- the analysis and interpretation new variant is difficult
- Expensive equipment[28]

	cfDNA
Sensitivity	85.0%
Specificity	99.6%
Accuracy	99.3%

Analytical and Clinical Validation of a Digital Sequencing Panel for Quantitative, Highly Accurate Evaluation of Cell-Free Circulating Tumor DNA(2015)

SequensingTM

- high-quality sequencing of circulating tumor DNA
- over 50 cancer-related genes with a simple blood test
- reduction in the costs, complications
- invasive tissue biopsies for genomic testing

A plasmonic nanosensor with inverse sensitivity

- inverse sensitivity
- CTAB coated AuNRs
- interact with dsDNA

✓concentration-dependent AuNR aggregation

✓UV-Vis spectroscopy

✓Fast and easy to use

✓not involve enzymes

A plasmonic nanosensor with inverse sensitivity

- Simpler
- few experimental times
- few multi-step proceduces[24]

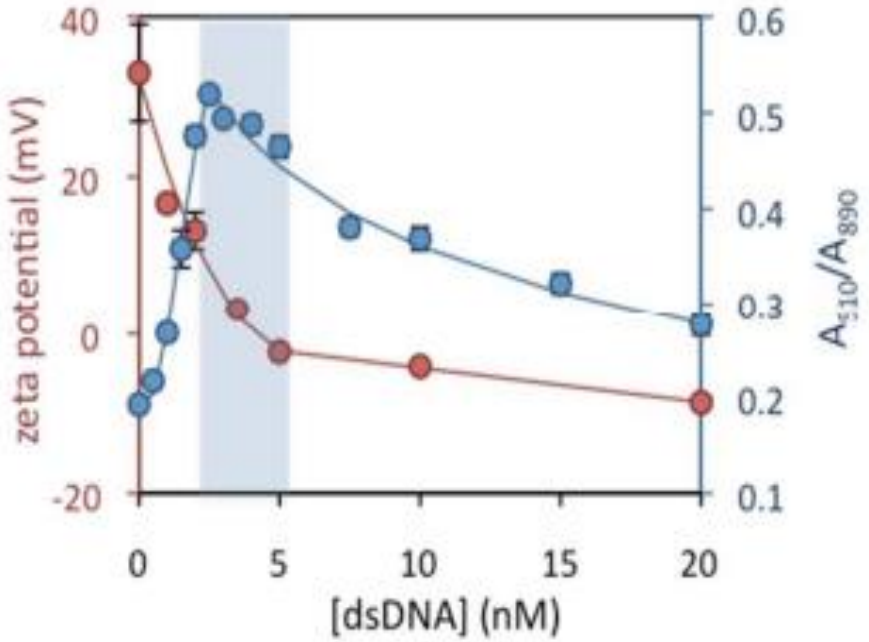
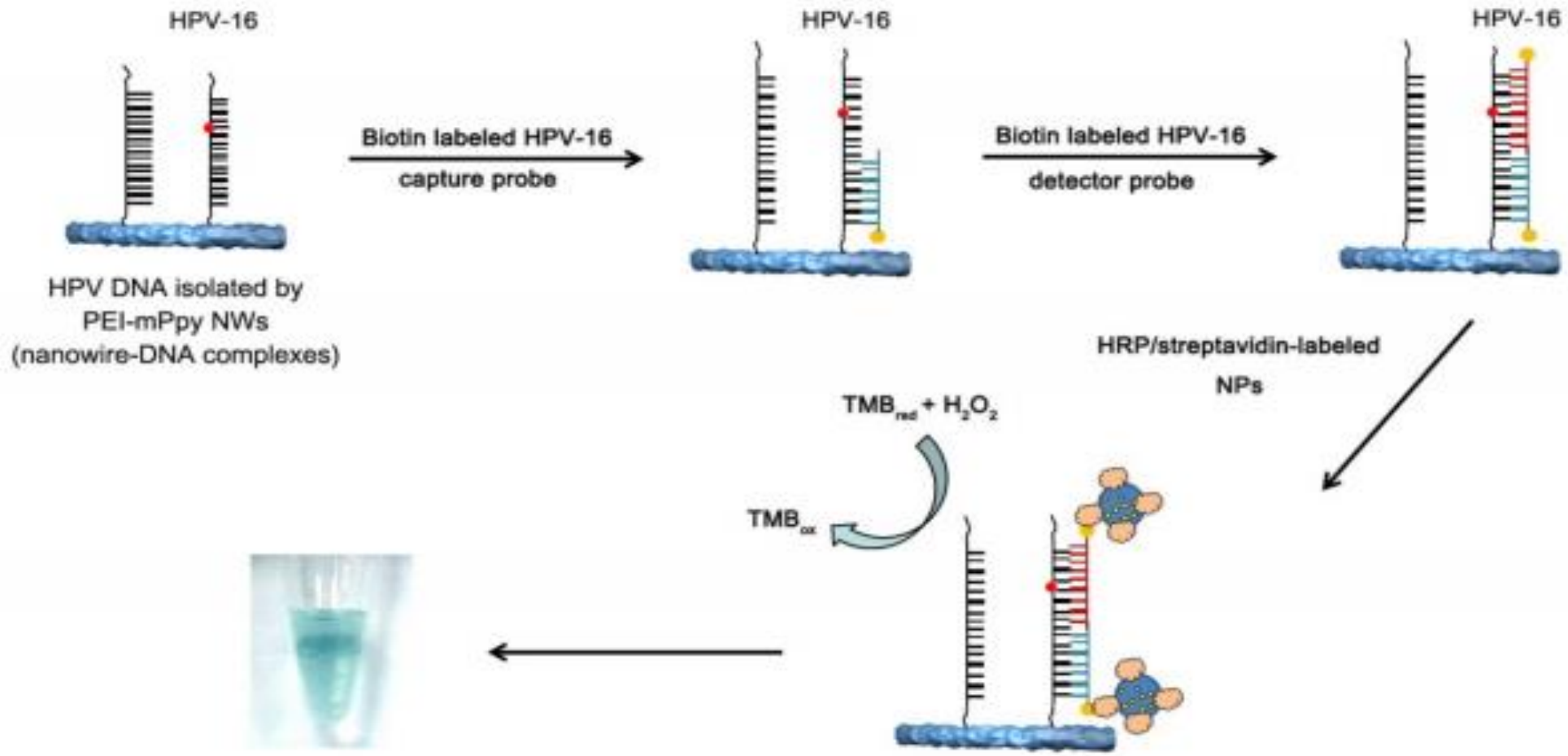


Fig. 3 Comparison between the effect of dsDNA concentration on the AuNR zeta potential (red) and the A_{510}/A_{890} (blue). The region of concentrations with higher AuNR aggregation is highlighted in pale blue.

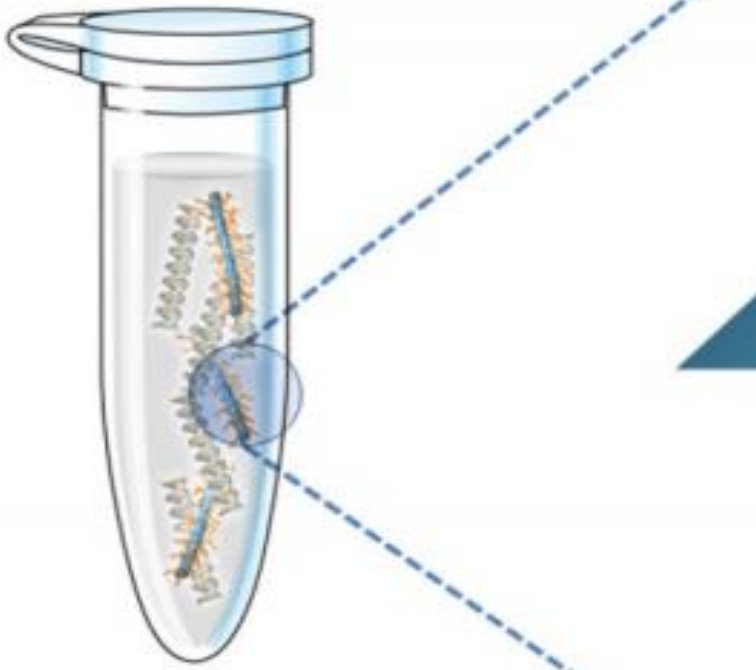
A Versatile Nanowire Platform

- Detection HPV
- minimal amounts of urine samples
- PEI-mPpy NWs
- extraction, identification, and PCR-free colorimetric detection
- cost-effective
- high sensitivity and accuracy
- nanowire-DNA complexes
- Inexpensive[30]

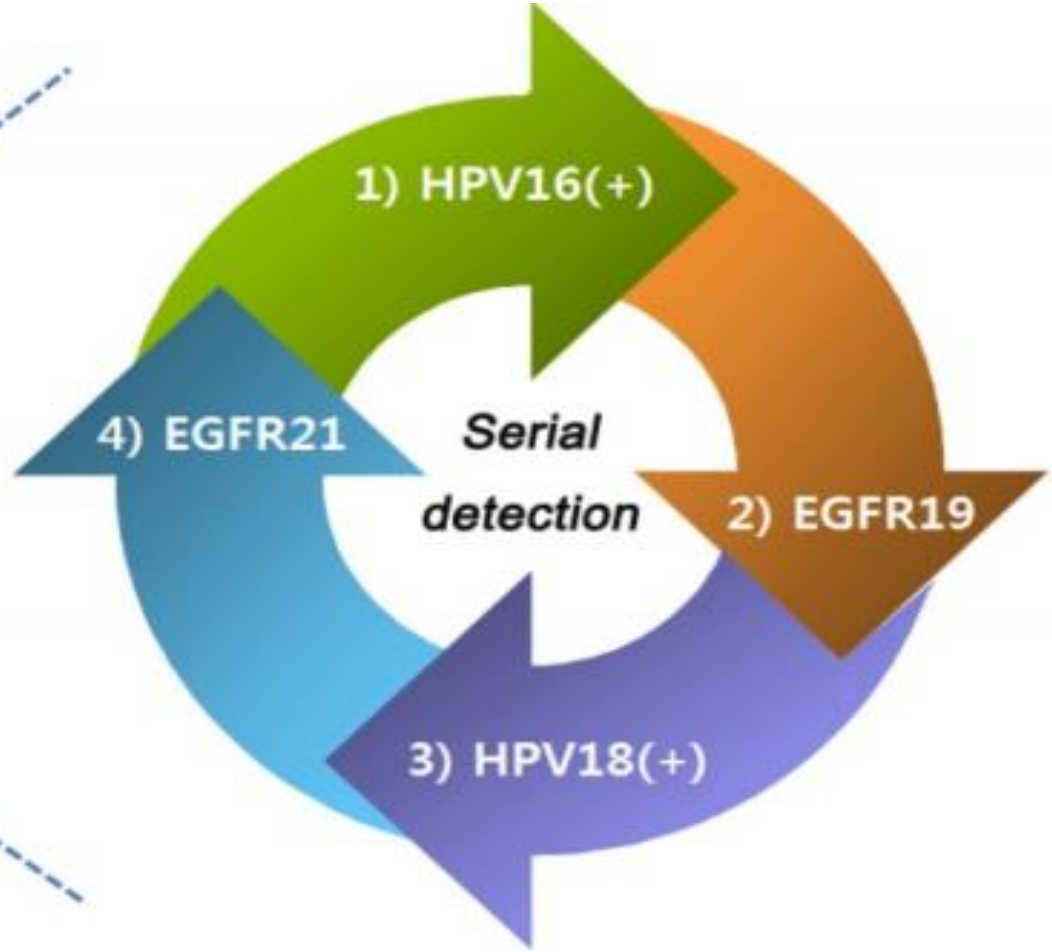
A Versatile Nanowire Platform



A Versatile Nanowire Platform



HPV DNA isolated
by PEI-mPpy NWs
(nanowire-DNA complexes)



A Versatile Nanowire Platform

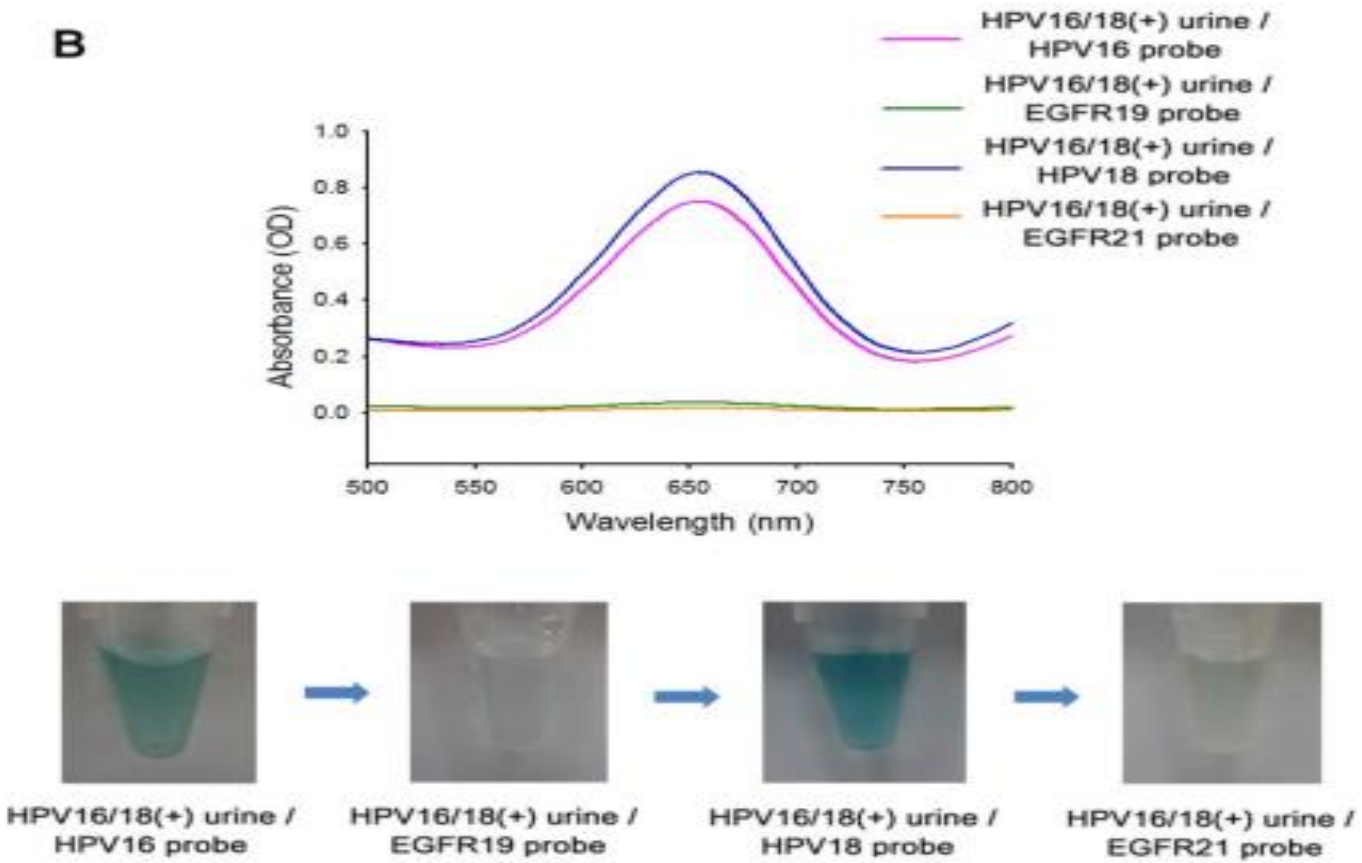


Figure 6. (a) A novel approach of PEI-mPpy NWs in the extraction, identification, and PCR-free sequential detection of multiple HPV genotypes from urine specimens of cervical cancer patients. (b) The UV-Vis colorimetric results of cfDNA isolated by PEI-mPpy NWs from urine of cervical cancer patients who were found to be positive for both HPV16 and HPV18, demonstrating multiple uses of the same nanowire-DNA complexes for the detection of HPV with different genetic variations. However, no response was observed for non-HPV probes (EGFR19 and EGFR21). The photographs indicate the color change as a result of type-specific hybridization between target HPVs and their complementary probes.

electroactive conducting polymer nanowire platforms

Use of electroactive conducting polymer nanowire platforms

- the efficient capture and release
- plasma samples from patients with breast and lung cancers.
- at high yield and purity
- simple, fast, and reliable
- accurate diagnostic and prognostic information
- yielding improved outcomes for cancer patients
- interactions between Ppy/Au NWs and DNA with pico green fluorescence[31]

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Thank you!